criteria. Any initial disagreement was resolved by discussion or consensus with a third reviewer where necessary.
Inclusion criteria were: a) randomised controlled trials (RCT), prospective cohort studies (PCS) or case control studies (CCS); b) including adults over 45 years old with knee pain or OA; c) undertaking exercise or physical activity explicitly over at least three months; and d) measuring a safety related outcome including adverse events, pain, physical functioning, radiographic or magnetic resonance imaging (MRI) OA biomarker progression or progression to total knee replacement (TKR). Included studies were assessed for risk of bias using the Cochrane risk of bias tool for RCT and the Quality In Prognosis Studies risk of bias tool for PCS and CCS. Safety data were extracted and analysed by narrative synthesis.
Results: In total, 8605 unique references were identified by the search and 37 primary studies were included in the review. The included studies were made up of 35 RCT, 1 PCS and 1 CCS, which were undertaken in 16 different countries and were of variable quality. In total, 7194 older adults with either knee pain or clinical, radiographic and combined OA diagnoses were included. The 35 RCT investigated a variety of exercise and physical activity interventions including: walking, Tai Chi, aerobics, strengthening, flexibility, balance, and exercise in water, of between three months and two years duration. 15 studies provided data on adverse events, 35 on pain, 32 physical functioning and 5 some form of radiographic or MRI biomarker of OA progression. In terms of safety results, only eight serious adverse events were reported: one inguinal hernia, five falls (one of which resulted in a head laceration injury and one a fractured radius), one participant fractured her foot as a result of dropping a weight and one had an exacerbation of existing back pain. Mild adverse events of increase in knee pain occurred in a minority of individuals in eight RCT. There was no evidence of a statistically significant increase in pain, decrease in physical function, or deterioration in radiographic or MRI findings (compared to either control group at follow up or within the same group comparing baseline to post treatment data). The single, moderate risk of bias, CCS investigated factors associated with OA progression to TKR in Finnish older adults. It concluded that increasing levels of regular exercise were associated with less chance of progression when compared to those who did not carry out regular exercise. The single, high risk of bias, PCS investigated occupational physical activity exposure in older adult members of a French energy company. From a set of 10 physical activity variables, only prolonged exposure to kneeling and squatting activities (between 1 and 25 years) was associated with severe knee pain (Adjusted OR 1.4 95% CI 1.04, 1.85). Overall risk of bias across studies was variable with performance, attrition, and reporting bias frequently being unclear.
Conclusions: This systematic review demonstrates that long-term exercise and physical activity, lasting three months or more, is safe for older adults with existing knee pain or OA. Serious adverse events with exercise are very rare. However, the risk of bias of included studies was often a concern.
These findings match existing expert consensus regarding the safety of exercise and current recommendations for exercise and physical activity as "core" treatments in clinical guidelines.

10
BIOACTIVITY IN AN AGGRECAN 32MER FRAGMENT IS MEDIATED VIA TOLL-LIKE RECEPTORS

A.J. Fosang †, S.B. Golub †, K. Last †, S. Lees †, R. Wilson ‡, A. Aspberg §, C.B. Little ‖, P. Sutton †. † *Univ. of Melbourne & Murdoch Childrens Res. Inst., Melbourne, Australia;* ‡ *Univ. of Tasmania, Hobart, Australia;* § *Univ. of Copenhagen, Copenhagen, Denmark;* ‖ *Univ. of Sydney & Kolling Inst., Sydney, Australia*

Purpose: One naturally-occurring fragment of aggrecan catabolism is the 32mer fragment derived from the aggrecan interglobular domain following cleavage by both ADAMTS and MMP enzymes. The aim of this study was to determine whether the aggrecan 32mer fragment was bioactive, and if so, to elucidate the mechanism.
Methods: Mouse femoral head cartilage explants, epiphyseal chondrocytes, synovial fibroblasts or peritoneal macrophages were cultured for 24 hours in the presence of synthetic mouse 32mer (FFGVGGEDDITIQTVTWPDLELPLPRNVTEGE; Auspep, Australia) or a scrambled peptide comprising the same amino acids in a random order. Global gene expression in explants treated with IL-1α or 32mer was compared on Illumina mouse W6-6_V2 expression beadchips. Thereafter, changes in gene and protein expression were validated in cell and explant cultures by qPCR, IL-6 ELISA and gel zymography.
Results: In the microarray experiment, 32mer-treated cartilage explants decreased their expression of genes encoding matrix proteins and increased their expression of pro-inflammatory/pro-catabolic genes. The results showed that the pattern of genes that were up- and down-regulated by IL-1α and 32mer treatment were strikingly similar. Validation by qPCR confirmed that the 32mer, but not a scrambled 32mer peptide, regulated gene expression in articular chondrocytes, peritoneal macrophages and synovial fibroblasts from wildtype mice. The levels of IL-6 measured by ELISA and gelatinase activity measured by gel zymography were also significantly increased in cells treated with 32mer or IL-1α, but not the scrambled peptide. The striking correlation in the pattern of genes regulated by IL-1α and 32mer treatment raised the possibility that IL-1α and the 32mer might share components of a signalling pathway; for example MyD88 is an essential adaptor protein for both the IL-1 receptor and most Toll-like receptors (TLR). We therefore compared cells from wildtype mice and MyD88 null mice for the effects of 32mer stimulation and found that neither gene expression nor levels of IL-6 cytokine were increased in cells harvested from mice lacking MyD88, suggesting that the 32mer might be an activator of TLR signalling. We next compared the effect of 32mer treatment on macrophage cell lines derived from wildtype, TLR2 or TLR4 null mice and found that whereas stimulation with the 32mer was identical in wildtype and TLR4 null cells, the 32mer failed to signal in TLR2 null cells. These results strongly suggest that the aggrecan 32mer is a ligand for TLR2. We next used complementary hydropathy to derive an antigenic sequence for raising an antibody (αCG11) against a putative 32mer-binding protein, then used αCG11 immunoaffinity chromatography to isolate the putative 32mer-binding protein from 4M GuHCl extracts of human OA and juvenile cartilage. Mass spectrometry analyses identified PRELP, a small leucine-rich repeat proteoglycan, as the major molecule binding to and eluting from the immunoaffinity column in both samples, and this was confirmed by Western blotting with αPRELP antibodies. Decorin and biglycan are also members of the small leucine-rich repeat proteoglycan family; they are related to PRELP, and both have been identified as TLR2 and TLR4 ligands. Further experiments to resolve the biological role of PRELP in 32mer-mediated TLR activation in chondrocytes, synovial fibroblasts and macrophages are in progress.
Conclusions: These studies identify the aggrecan 32mer as an endogenous activator of TLR2-mediated immunity with the potential to accelerate inflammation and cartilage destruction *in vivo*.

11
DECORIN-DEFICIENT MICE ARE LESS PRONE TO DEVELOP OSTEOARTHRITIS AFTER FORCED EXERCISE

T. Gronau †, U. Hansen ‡, D.G. Seidler ‡, R.V. Iozzo §, A. Aszódi ‖, C. Prein ¶, H. Clausen-Schaumann ¶, K. Krüger #, F.C. Mooren #, J. Bertrand †, T. Pap †, P. Bruckner ‡, R. Dreier ‡. † *Inst. of Experimental Musculoskeletal Med., Univ. Hosp. Münster, Münster, Germany;* ‡ *Inst. for Physiological Chemistry, Univ. Hosp. Münster, Münster, Germany;* § *Depatment of Pathology, Thomas Jefferson Univ., Philadelphia, PA, USA;* ‖ *Experimental Surgery and Regenerative Med., Ludwig-Maximilians-Univ. Munich, Munich, Germany;* ¶ *Dept. of Applied Sci. and Mechatronics, Munich Univ. of Applied Sci., Munich, Germany;* # *Inst. of Sports Med., Justus-Liebig Univ. Giessen, Giessen, Germany*

Purpose: Here we analyzed the impact of decorin-deficiency (Dcn-/-) on the biomechanical properties of cartilage and the osteoarthritis development in a mouse model via forced exercise on a treadmill. In articular cartilage, the small leucine-rich proteoglycan decorin (Dcn) is located within the interterritorial regions of the extracellular matrix (ECM), where it is associated with thick, well-banded collagen fibrils. Inside the ECM, decorin binds and inactivates TGF-β and thereby regulates the activity of this cytokine, which is known to influence the sulfation of glycosaminoglycan (GAG) chains.
Methods: Osteoarthritis (OA) was induced in three month-old Dcn-/- and WT mice via forced running on a treadmill and the severity was scored by a modified Mankin score. To analyze biomechanical properties, knee cartilage of Dcn-/- mice and wild-type (WT) mice was examined at different developmental stages by histology, immunohistochemistry, and atomic force microscopy (AFM). The amount of active TGF-β1 in the supernatant of cultured Dcn-/- and WT chondrocytes was analyzed by ELISA. Expression of GAG modifying enzymes was assessed by semi-quantitative RT-PCR.

Results: Both genotypes exhibited osteoarthritic changes of the articular cartilage after six weeks of forced running on a treadmill. However, the changes were less severe in Dcn-/- compared to WT mice (Mankin score 4.5 (Dcn-/-) versus 6.5 (WT)). In juvenile and adult, but not newborn Dcn-/- mice, staining of cartilage for highly sulfated GAGs via Alcian blue (pH 1.0) revealed an increased intensity compared to WT mice. Likewise, the use of an antibody against chondroitin-4-sulfate (C4S) stubs (Δ) showed a comparably enhanced staining pattern in Dcn-/- mice. In contrast, we found no differences in staining for proteoglycan core proteins (e.g. aggrecan, biglycan, versican) or collagens (II, VI, IX, X). At the mRNA-level, Dcn-/- mice exhibited an increased expression of PAPS-synthase-1 (PAPSS-1), chondroitin-4-sulfotransferase-1 (C4ST-1) and chondroitin-4-sulfotransferase-2 (C4ST-2), which are involved in GAG modification. This was also true at the protein level as visualized via staining for PAPSS-1 and ΔC4S. Moreover, AFM analysis of articular cartilage sections of three months-old mice revealed an increased compressive stiffness of decorin-deficient articular cartilage. Those abnormalities were accompanied by enhanced levels of active TGF-β1 in decorin-deficient chondrocytes in vitro.

Conclusions: We propose the following mechanism of OA-attenuation in Dcn-/- mice: Decorin sequesters the cytokine TGF-β in the extracellular matrix. Higher levels of active TGF-β in cartilage of Dcn-/- mice are likely to prevent inappropriate chondrocyte hypertrophy and, hence, induction of osteoarthritis. TGF-β also triggers increased sulfation of GAG chains, via an upregulation of PAPSS-1, C4ST-1 and C4ST-2 and consequently a stiffer cartilage matrix. Therefore, decorin-deficient animals are less prone to develop osteoarthritis due to a combination of elevated levels of TGF-β-activity and enhanced compressive stiffness of the tissue.

12
CTGF IS RELEASED FROM THE PERICELLULAR MATRIX OF ARTICULAR CARTILAGE UPON MECHANICAL INJURY, AND CONTROLS THE BIOAVAILABILITY OF TGFβ

X. Tang, C. McLean, A. Chanalaris, T.L. Vincent. *Univ. of Oxford, Oxford, United Kingdom*

Purpose: Work from our lab has identified an important role for the pericellular matrix (PCM) in the mechanosensitive release of sequestered regulatory molecules such as FGF2. (Vincent et al. 2007). A proteomic analysis of PCM proteins also identified connective tissue growth factor (CTGF also known as CCN2). CTGF is significantly elevated in osteoarthritis (OA) (Hermansson et al. 2004) but its role in cartilage is obscure. We investigated the control of the release of CTGF in cartilage, and its biological function.

Methods: His-tagged human recombinant CTGF protein was stably expressed in HEK293 cells and purified using nickel affinity chromatography. To look for novel gene targets a microarray study was performed on isolated human articular chondrocytes (HACs) with or without CTGF stimulation. Target genes were validated by real time PCR. Phosphorylation of SMADs was examined by western blot. The dependence of SMAD phosphorylation and gene regulation on TGFβ was examined using a TGFβ neutralising antibody and the TGFβ type I receptor inhibitor SB431542. For injury studies, porcine cartilage was either explanted into serum free DMEM for up to 24h, or was rested (48 hours) and then re-cut in fresh serum free DMEM for different time periods. Both explantation and re-cut conditioned media were collected and analysed under either reducing or non-reducing conditions. Articular cartilage was examined by confocal microscopy for CTGF and the latency associated peptide of TGFβ (LAP).

Results: In the microarray study, we identified four genes that were strongly up-regulated by CTGF in human chondrocytes. All genes were known to be TGFβ inducible and their expression showed a similar temporal pattern to TGFβ. CTGF was able to activate SMAD2 in porcine chondrocytes. Both CTGF induced gene expression and SMAD2 phosphorylation was abrogated by TGFβ neutralising antibody and the receptor inhibitor SB431542, suggesting that CTGF activity was TGFβ dependent. Adding exogenous CTGF to rested porcine cartilage for 1 or 4 hours, led to increased TGFβ protein in the medium but no significant change in the mRNA level of TGFβ. Injury to porcine cartilage by explantation or re-cutting caused rapid massive accumulation of endogenous CTGF in the conditioned medium (within 5 minutes), followed by a delayed accumulation of TGFβ (from 1 hour). The accumulation of both proteins in response to injury occurred in the presence of cycloheximide, suggesting proteins were released from a pre-formed pool rather than by new protein synthesis. When we analysed the explantation conditioned medium under non-reducing condition, CTGF was found to migrate at 150KDa along with components of the latent TGFβ complex (LAP). Although TGFβ was not evident by western blot under non-reducing conditions, it was clearly present when the same medium was run under reducing conditions suggesting that TGFβ was also tied up in the high molecular weight complex. This was supported by the observation that the explantation conditioned medium was able to activate SMAD2, and this activity resided in a high molecular (>50kDa) fraction. Confocal microscopy revealed that CTGF was present in the pericellular matrix as was the latent complex of TGFβ.

Conclusions: Our results show that pericellular CTGF, like FGF2, is released from the matrix upon cartilage injury. The latent complex of TGFβ is also in the pericellular matrix and released with CTGF upon injury. The temporal sequence suggests that CTGF is released first and then contributes to the release of latent TGFβ, although it is possible that both CTGF and TGFβ are already bound and are released simultaneously upon injury. Either way we have described a novel pathway by which cartilage injury controls the bioavailability of TGFβ through matrix bound CTGF.

13
GALECTIN 3 HAD A PROTECTIVE ROLE IN MURINE OSTEOARTHRITIS INDUCED BY MENISCECTOMY AND AGING

N. Hafsia. *Unite 606 Inserm, Paris, France*

N Hafsia 1; F Poirier2; D Delacour 2; P Reboul 3; D Côme 1; P Van Lent 4; M Cohen-Solal 1; F Lioté 1; HK Ea1

Purpose: Osteoarthritis (OA) is the most common adult joint disease characterized by cartilage destruction, subchondral bone remodelling and mild synovial inflammation. Pathogenesis of OA involves mechanic, age and genetic factors. Galectin 3 (Gal3) expression is increased in OA cartilage and synovial fluid. Gal3 is involved in multiple cellular functions including proliferation, differentiation and cell death. During embryogenesis, the growth plate of Gal3 mutant mice has a reduction of hypertrophic zone and many lacunae due to a loss of chondrocytes. These results suggest a role of Gal3 in chondrocyte differentiation. The aim of this study is to determine the role of Gal3 in osteoarthritis and to assess its functions in chondrocyte biology.

Methods: *In vivo*, murine OA was induced in Wild type (WT) 129 and Gal3 mutant mice by partial medial meniscectomy (MNX) of the right knee. A sham operation was performed on the left knee. Animals were sacrificed 4 weeks after surgery. Cartilages lesions were assessed by according to OARSI recommendations. Chondrocyte apoptosis was assessed by TUNEL labelling and chondrocyte catabolism by immunostaining for aggrecanases and metalloproteases. Subchondral bone modifications were evaluated by microCT. In parallel, we assessed joint lesions durinbg ageing in 14 month old mice. In vitro, primary murine chondrocytes, isolated from new born WT and Gal3 mutant mice, were cultured and stimulated by inflammatory cytokines (IL-1β and TNF-α). Anabolism, catabolic and differentiation responses were assessed by RT-qPCR.

Results: *In vivo*, MNX induced in Gal3 mutant mice more severe OA cartilage lesions compared to WT as assessed by OARSI scoring (4.43 ± 1.02 vs 1.30 ± 0.77, $p<0.05$). Expression Gal3 decreased in WT cartilages after MNX compared to sham operated cartilage ($34.5\% \pm 4.9$ vs $53\% \pm 2.8$; $p<0.01$). Cartilages lesions were associated with higher ratio of chondrocyte positive-TUNEL staining in Gal3 mutant cartilages compared to WT ($17.7\% \pm 6.2$ vs $10.6\% \pm 3.3$ $p<0.02$). Similarly, immunostaining for ADAMTS-5 ($26.7\% \pm 0.5$ vs $16\% \pm 1.4$, $p<0.02$) and VDIPEN ($52\% \pm 4$ vs $30\% \pm 2$; $p<0.05$) was increased in Gal3 mutant cartilages. Moreover, collagen type X expression was increased in Gal3 mutant cartilage. No difference was observed in subchondral bone modifications. Finally, Gal3 mutant mice developed spontaneous OA cartilage lesions at 14 months old. In vitro, IL-1β stimulation increased ADAMTS-4(4.58 ± 1.97 vs; 1.60 ± 0.28 ; $p<0.05$) and ADAMTS-5 (4.52 ± 2.19 vs 1.78 ± 1 ; $p<0.05$)mRNA expressions in Gal3 mutant chondrocytes compared to WT chondrocytes.

Conclusion: Gal3 deletion exacerbated murine OA lesions induced by mechanical stress and by ageing. OA lesions were associated with increased chondrocyte catabolism, differentiation and death. These results suggested Gal3 had a protective role in OA and chondrocyte homeostasis.